YYÜ TAR BİL DERG
(YYU J AGR SCI)
2012, 22 (1):33-39

Geliş Tarihi (Received): 30.05.2011
Kabul Tarihi (Accepted): 15.01.2012

Araştırma Makalesi/Research Article (Original Paper)

Modeling of the Effect of Potassium Bicarbonate on Sclerotial Germination of *Sclerotinia sclerotiorum* in vitro by Multi Regression Analysis

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Abstract: In this study, we evaluated to effect of days and different doses of potassium bicarbonate ($KHCO_3$) on sclerotial germination of *Sclerotinia sclerotiorum* by multiple regression analysis. The equation produced for sclerotial germination of *S. sclerotiorum* was derived as affected by doses and days. As a result of ANOVA and multiple regression analysis, it was found that there was close relationship between actual and predicted sclerotial germination of *S. sclerotiorum*. The produced prediction models in the present study are SG= (a) - (b x Dose) + (c x Time) where SG is sclerotial germination of *S. sclerotiorum*, a, b and c are co-efficiencies. R^2 value was found as 0.80.

Keywords: Modeling, sclerotial germination, white rot, $KHCO_3$

Çoklu Regresyon Analizi ile *Sclerotinia sclerotiorum* Sklerotlarının Çimlenmesi Üzerine Potasyum Bikarbonat'ın Etkisinin Modellemesi

Özet: Bu çalışmada çoklu regresyon analizi ile farklı potasyum bikarbonat ($KHCO_3$) dozları ve zamanın *Sclerotinia sclerotiorum*'un sklerot çimlenmesi üzerine etkileri değerlendirilmiştir. *S. sclerotiorum*'un sklerot çimlenmesi için üretilen eşitliğin zaman ve dozlar tarafından etkilediği görülmüştür. ANOVA ve çoklu regresyon analizi sonucuna göre *S. sclerotiorum*'un sklerot çimlenmesinin gerçek ve tahmini değerleri arasında yakın bir ilişki bulunmuştur. Elde edilen tahmini modelde SG= (a) - (b x doz) + (c x zaman), SG *S. sclerotiorum* sklerot çimlenmesi, a, b ve c katsayılardır. R^2 değeri 0.80 olarak bulunmuştur.

Anahtar Kelimeler: Modelleme, Sklerot çimlenmesi, Beyaz çürüklük, $KHCO_3$

Introduction

The soil borne fungi, agents of root rot and damping off diseases, cause important economic losses on various crops that often results in the death of plants every year. *Sclerotinia sclerotiorum* (Lib.) de Bary is an aggressive and destructive fungal pathogen that causes rot root and wilting on various host plants (Bolton et al. 2006; Dillard and Cobb 1995). The fungus survives in soil as sclerotia, which are hardened structures of fungal mycelium (Ben-Yephet et al. 1993; Yangui et al. 2008) and a viable source of inoculum for long periods under unfavorable conditions (Wu and Subbarao 2006; Bae and Knudsen 2007).

Synthetic fungicides are used to control soil borne pathogens on vegetable growing areas at all world. In recent years, public demands to reduce pesticide use, stimulated by greater awareness of environmental and health issues as well as the development of fungicide resistant strains of pathogens, have created the need to find alternatives to pesticides. Natural substances such as bicarbonates and their salts may be used to achieve this aim. The main advantages of using bicarbonates compared with fungicides include their relatively low mammalian toxicity, a broad spectrum of modes of action and relatively low cost (Olivier et al. 1998). They have been shown to be effective growth inhibitors of some soil borne fungal pathogens (Ricker and Punja 1991; Arslan et al. 2009; Ordonez et al. 2009).

Models are commonly explored using computational and/or simulation techniques (Odabas et al. 2009a; Odabas et al. 2010; Caliskan et al. 2010). The simulation software may be general-purpose, intended to capture a variety of developmental processes depending on the input files, or special-purpose, intended to capture a specific phenomenon. Input data range from a few parameters in models capturing a fundamental mechanism to thousands of measurements calibrated descriptive models of specific plants (species or individuals). Standard numerical outputs (i.e. numbers or plots) may be complemented by computer-generated images and animations (Odabas et al. 2009b). Most of the researches have investigations focused on plant developmental periods from seed sowing to reproductive stages and from reproductive stages to harvest (Celik and Odabas 2009).

There are applied models relevant to epidemiological analyses of some soil borne pathogens. Quantifying the relationships and effects of pathogen, plant, and environmental factors on disease development by means of quantitative models can help in the design and efficient use of management strategies for soil borne pathogens (Clarkson et al. 2004; Navas-Cortés et al. 2007). Clarkson et al. (2004) were evaluated the feasibility of developing a prediction model for the carpogenic germination of *S. sclerotiorum* sclerotia in the field based on their response to temperature and soil water potential. Additionaly, Navas-Cortés et al. (2007) determined to quantify the combined effects of biotic (a range of virulence, inoculum density, and cultivar susceptibility) and abiotic (soil temperature) factors on development of Fusarium wilt on chickpea.

To our knowledge, there is not much study on mathematical modeling for sclerotial germination of *S. sclerotiorum*. The objective of this study was developed model of estimating the sclerotial germination of *S. sclerotiorum in vitro* exposure to increased doses of $KHCO_3$ at different days by multi linear regression.

Materials and Methods

Fungal culture and production of sclerotia

S. sclerotiorum isolate (TR-Ss-07) was originally obtained from bean growing areas in Bafra, Samsun province during routine disease surveys in 2009. This isolate was isolated from diseased bean plants showing signs of white rot and identified according to its cultural features. Culture of *S. sclerotiorum* isolate was maintained on potato dextrose agar (PDA: Oxoid). The PDA slants were stored at 4^0C and served as stock cultures for further use. Then, sclerotia were obtained from pure cultures of *S. sclerotiorum* that were grown on PDA. Sclerotia were collected, placed in sterile microtubes, and kept at 4°C until they were used.

Assessment of sclerotial germination

Different potassium bicarbonate concentrations on the following basis: 0, 2, 4, 6, 8, 10, 25, 50, 75, or 100 mM ($KHCO_3$, Carlo Erba Reagenti (Milan-Italy) were added to autoclaved and cooled PDA medium at 50^0C for *S. sclerotiorum.* The medium was dispensed aseptically into 9-cm-diameter petri plates. Obtained sclerotia were surface disinfested to avoid proliferation of bacterial contaminants by placing them in 70% ethanol for 40 s, and rinsed in sterile-distilled water (SDW), adding a solution of streptomycine (200 µg/ml), and keeping them at 4°C overnight and blotted dry on sterile paper towels to eliminate excess antibiotic solution. Then, once dried, ten sclerotia were placed on 9-cm diameter petri dishes containing PDA with the concentrations of $KHCO_3$ (Ordonez et al. 2009). The Petri dishes were incubated at 25 ^{0}C for 7 days, and the number of germinated sclerotia was daily recorded for each treatment. All experiments were conducted twice.

Experimental design and data analyses

All experiments were conducted in a complete randomized design with ten treatments and four replications. Analysis of variance was implemented using the program *Minitab* (version 12, “Minitab”, USA) and Duncan at 0.05 significance level was used to compare treatment means.

Model Construction

Multiple regression analysis of the data obtained from in vitro study was executed for sclerotial germination of *S. sclerotiorum*. The general purpose of multiple regression is to learn more about the relationship between several independent and a dependent.

The linear regression model assumes that the relationship between the dependent variable y_i .The y_i is called the *dependent variable*. The decision as to which variable in a data set is modeled as the dependent variable and which are modeled as the independent variables may be based on a presumption that the value of one of the variables is caused by, or directly influenced by the other variables. The x_i are called *independent variables*. The r x_i may be viewed either as random variables, which we simply observe, or they can be considered as predetermined fixed values which we can choose. β is a *p*-dimensional *parameter vector*. Its elements are also called *effects*, or *regression coefficients*. Statistical estimation and inference in linear regression focuses on β. ε_i is called the *error term*. This variable captures all other factors which influence the dependent variable y_i other than the regressor x_i. The relationship between the error term and the regressor, for example whether they are correlated is a crucial step in formulating a linear regression model, as it will determine the method to use for estimation (Weisberg 2005). A search for the best model to predict the sclerotial germination was conducted with various subsets of the independent variables, namely, dose (mM) and time. The best estimating equation for the sclerotial germination (SG) of *S. sclerotiorum* was determined with the R-program and formulized as SG= (a) - (b x Dose) + (c x Time) where SG is sclerotial germination of *S. sclerotiorum*, a, b and c are co-efficiencies of the produced equation (Table 1). Multiple regression analysis was carried out until the least sum of square (R^2) was obtained. 3-D graphics were performed by *Slidewrite* program.

Result

The increasing concentrations of potassium bicarbonate applications affected sclerotial germination of *S. sclerotiorum.* At 48 h sclerotial germination of it was determined from 0 to 6 mM, whereas at 24 h sclerotial germination did not observed neither treatment. But 100% germination was occurred at control after fourth days (Fig. 1). Also, at the end of 7 days all sclerotia germinated at concentration from 0 to 25 mM while sclerotia germination did not occur in maximum $KHCO_3$ concentration (100 mM)

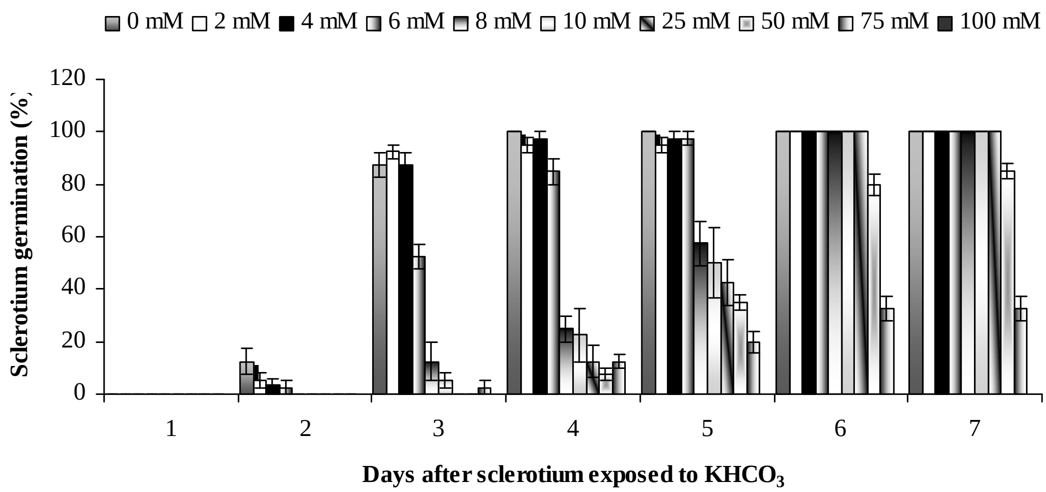

Figure 1. Germination of the sclerotium (%) of *Sclerotinia sclerotiorum* exposed to different concentrations of potassium bicarbonate ($KHCO_3$) after seven days *in vitro.* Bars ± standard error (*n* = 4).

As a result of the analysis the effects of doses and days on sclerotial germination of *S. sclerotiorum* have been found significant and an equation has been formed. Regression statistics are provided in Table 1. With regard to the regression statistics belonging to the sclerotial germination, it is observed that R^2 is 0.80. ANOVA significance F value has shown the validity of the model in other words whether or not the model can be formed. In this study as this value is below 1%, the result of analysis has a significance of 1%. Following the determination of the importance level, mathematical equation has been obtained by using co-efficients and corresponding independent x variable (SG) and dependent y variables (dose and day).

Table 1. Regression statistics of sclerotial germination of *Sclerotinia sclerotiorum*

Regression Statistics	
Multiple R	0,801398
R Square	0,803124
Adjusted R Square	0,639655
Standard Error	2,555048
Observations	280

ANOVA

	Df	*SS*	*MS*	*F*	*Significance F*
Regression	2	3246,236	1623,118	248,6291	1,49E-62
Residual	277	1808,331	6,528272		
Total	279	5054,568			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	0,224191	0,364691	0,614743	0,539229	-0,49373	0,94211
dose	-0,0612	0,004577	-13,3705	8,48E-32	-0,07021	-0,05219
day	1,3625	0,076347	17,84623	6E-48	1,212207	1,512793

Mathematical model for sclerotial germination has been formed as a - (b x Dose) + (c x Day). In this formula a, b and c symbolizes the co-efficient obtained as a result of multi regression analysis. By taking into consideration the co-efficient in the regression statistics, sclerotial germination model of *S. sclerotiorum* has been formed.

Sclerotial Germination (SG) = (0.224) - (0.0612 x Dose) + (1.363 x Day)
Standard Error (SE) = 0.26*** $3.21E^{-3}$ *** 0.0626 ***
Regression co-efficient (R^2) = 0.80

The relation between the sclerotial germination of *S. sclerotiorum* corresponding to the real values and the approximate sclerotial germination obtained from mathematical equation has been shown in the Figure 2.

The other points represent the sclerotial germination obtained from the model. The closer these values are to reality, the higher R^2 value of the mathematical model. In this study R^2 value obtained (0.80) shows that a model with 80% close to the reality has been formed. The effects of doses and days on sclerotial germination of *S. sclerotiorum* have been shown in Figure 3. Mathematical equation has been benefited while showing this change caused by doses and days on the sclerotial germination of *S. sclerotiorum*. In this graphic (Figure 3) mesh part shows the change in the sclerotial germination throughout *S. sclerotiorum* with days and doses of $KHCO_3$. It is drawn with the help of mathematical equation obtained and *Slitewrite* graphic program. The most important feature of this program is to draw 3 dimension graphic by using not only the values entered but also the mathematical equation obtained. Figure 3 shows that the sclerotial germination of *S. sclerotiorum* increases as day's raises. In contrast, when the doses increase sclerotial germination decreases.

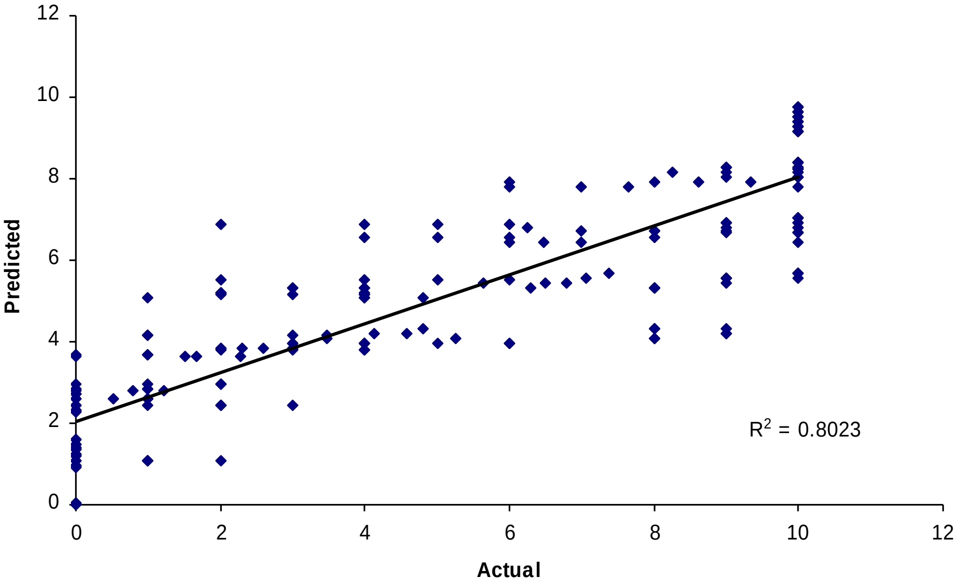

Figure 2. Relationship between actual and predicted the sclerotial germination of *Sclerotinia sclerotiorum*

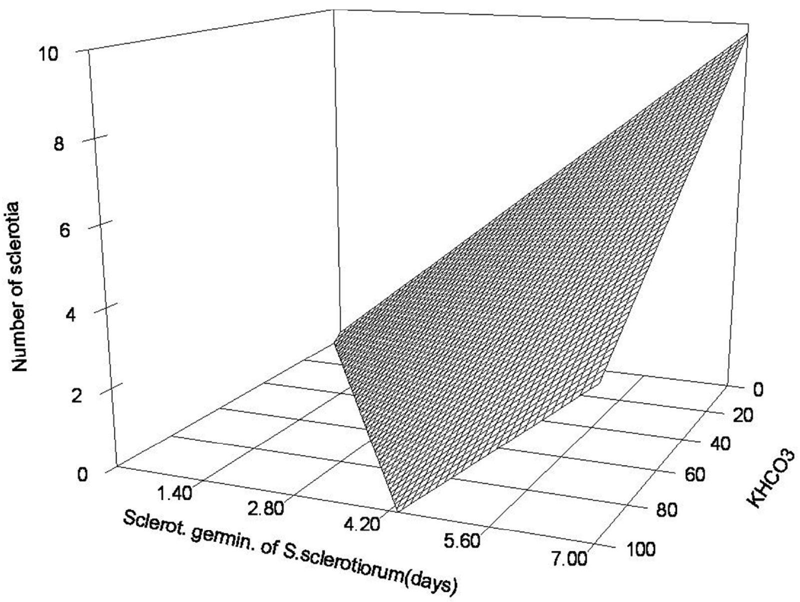

Figure 3. Sclerotial germination of *S. sclerotiorum* on petri dishes exposure to increasing concentrations of potassium bicarbonate ($KHCO_3$) on different days.

Discussion

Modeling the epidemiology of some soilborne pathogens were determined in the previous studies. Clarkson et al. (2004) were investigated the feasibility of developing a forecasting system for carpogenic germination of *S. sclerotiorum* sclerotia in the laboratory by determining key relationships among temperature, soil water potential, and carpogenic germination for sclerotia of two *S. sclerotiorum* isolates. As a result, it was determined that temperature showed a significant effect on both the rate of germination of sclerotia and the final number germinated. However, rate of germination was related to temperature according to a probit model was correlated positively with temperature and final number of sclerotia germinated. In addition, quantifying the relationships and effects of pathogen, plant, and environmental factors on disease development by means of quantitative models could be help in the design and efficient use of management strategies for root rot, wilt or damping-off disease caused by soilborne fungal pathogens. Navas-Cortés et al. (2007) were modeled the combined effects of soil temperature and inoculum density of *Foc*-0 and *Foc*-5 on disease developed in chickpea cvs. P-2245 and PV-61 differing in susceptibility to those races, using quantitative nonlinear models. In another studies, Lahlali et al. (2007) determinated to develop validated models predicting the *in vitro* effect of a_w and temperature on the radial growth of *B. cinerea*. However, there is no much a quantitative modeling approach for sclerotial germination of *S. sclerotiorum* which to be the one of the most important fungal pathogen. Additionally, in studies on mathematical modeling for sclerotial germination of *S. sclerotiorum* are limited. In present study, model of estimating the sclerotial germination of *S. sclerotiorum in vitro* exposure to increased doses of $KHCO_3$ at different times by multi linear regression was developed.

In our study, the model for estimating the sclerotial germination of *S. sclerotiorum* exposed to different doses of $KHCO_3$ at different times by multi linear regression were developed. Close relationship between actual and predicted sclerotial germination of *S. sclerotiorum* was determined. Using multiple regression equations it is very much likely to predict the variation in sclerotial germination of *S. sclerotiorum* as related to doses and days with high probability. In this study, R^2 value obtained (0.80) shows that model with 80% close to the reality has been formed for sclerotial germination of *S. sclerotiorum.* There are needed to apply models studies for epidemiological analyses of other important fungal pathogens. Additionally, quantifying the effects of environmental factors on fungal disease development by means of quantitative models can help in the design and efficient use of management strategies for soilborne pathogens.

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